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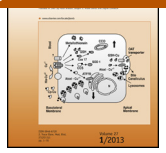

Pharmacology

Elements of margin of safety, toxicity and action of sodium selenite in a lipopolysaccharide rat model

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ABSTRACT

Project: Both septic shock and sodium selenite (Na_2SeO_3) lead to multiple organ failure through oxidation. Na_2SeO_3 has direct oxidant effects above the nutritional level and indirect anti-oxidant properties.

In a lipopolysaccharide (LPS) rat model we assessed margin of safety, toxicity and beneficial effect of pentahydrate Na_2SeO_3 ($5H_2O{\cdot}Na_2SeO_3$) at oxidant doses.

Procedure: In a three-step study on 204 rats we: (i) observed toxic effects of Na_2SeO_3 injected intraperitoneously (IP) and determined its Minimum Dose Without Toxic effect (MDWT) 0.25–0.35 mg/kg selenium (Se) content; (ii) injected IP LPS at 70% lethal dose (LD) followed, or not, one hour later by IP Na_2SeO_3 at MDWT and (iii) by doses > MDWT. At 48 h, in survivors, we measured plasma creatinine, lactate, aspartate and alanine aminotransferase (AST, ALT), nitric oxide (NO) and Se concentrations.

Results: (i) Na_2SeO_3 alone did not increase NO and lactate. Encephalopathy appeared at 1 mg Se/kg. Creatinine increased at 1–1.75 mg Se/kg, AST, ALT at 3–4.5 mg Se/kg, and the minimum LD was 3 mg Se/kg. (ii) Mortality after LPS was 37/50 (74%, [62–86%]) vs. 20/30 (67%, [50–84%]) when followed by Na_2SeO_3 at MDWT ($p = 0.483$) with a decreased in NO (−31%, $p = 0.038$) a trend for lactate decrease (−19%, $p = 0.068$) and an increased Se in plasma of survivals. (iii) All rats died at doses ≥0.6 mg/kg ($p < 0.001$).

Conclusion: Mechanisms of LPS and Na_2SeO_3 toxicity differ (i.e. NO, lactate). In septic shock $5H_2O{\cdot}Na_2SeO_3$ toxicity increased, margin of safety decrease, but IP administration of dose considered as oxidant of $5H_2O{\cdot}Na_2SeO_3$ showed beneficial effects.

Introduction

Septic shock, an uncontrolled systemic host response to invasive infection leading to multiple organ failure, is a major issue due to its frequency, cost and mortality rate [1]. Its physiopathology is better understood with increasing data supporting the key role of oxidant free radicals, especially on endothelium damage [2–5]. There is a growing interest in seleno-compounds in sepsis and more specifically in sodium selenite (Na_2SeO_3), however, if many studies have been performed on Na_2SeO_3 administration in septic shock patients [6], to our knowledge, no animal study has been done on its toxicity in septic animals, and few on mechanism of action [7]. In addition, Na_2SeO_3 is known to be a highly toxic compound leading to multiple organ failure and death [7–9]. Among the compounds containing selenium (Se), Na_2SeO_3 is one of the most toxic one due to its oxidant properties [9–11]. Oxidant properties of seleno-compounds are related to peripheral electronic structure shared between oxygen and Se (chalcogen family, or oxygen atom group) [8,12]. In Na_2SeO_3, the Se atom is under the Se^{4+} oxidation degree, which makes this molecule highly reactive especially on disulfur bridges [10,13–15]. In septic shock Na_2SeO_3 may act as an oxidant drug reducing the activity of overactivated polymorphonuclears through its cytotoxic properties and thus reducing the generalized

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overwhelming inflammation of septic shock [7,8,16–19]. In vitro, at concentrations higher than 2–5 μmol Se/L as Na_2SeO_3, it reduces the production of NO in activated cells by an inhibition of NFKB to DNA binding through oxidative mechanism [20]. It also induces cytotoxic effect especially in non-adherent cells and in activated cells [10,13,21]. This effect could be mediated by a toxicity of selenite on mitochondria on activated cells such as observed in cancer cells [13,15,21]. Results of a post-peritonitis sheep study are in favor of such an oxidant drug action in sepsis [22,23].

Clinical trials, including randomized multicenter study, administrating Na_2SeO_3 in septic shock patients have shown contradictory results, some of them showing a tendency for a decrease in mortality, especially those using a bolus administration [6,16,24–30]. The purpose of most of these studies was to deliver Se through selenite (Na_2SeO_3) administration, considering this administration as a mere Se supplementation. Indeed, most authors currently believe that Na_2SeO_3, even in bolus injection at doses largely above nutritional requirement, acts only as a source of Se required for selenoenzymes synthesis [6,25,27,28,30]. Selenoenzymes are one of the major antioxidant enzyme families in mammals [31–34] and there is a huge oxidative stress in septic shock by different mechanisms including the respiratory burst [2–5,35–38]. However, despite constant low plasma Se decrease, a tissue Se deficiency has not been shown in sepsis in any experimental study. In plasma, Se is present in selenoprotein-P (Sel-P) for about 60% and in plasma glutathione peroxidase (GPx-3) for about 30%. Plasma Se represents 0.5–1% of the 20–40 mg Se of the body [8,32]. One of the possible anti-oxidant actions for endothelium protection might be an induction of Sel-P. This mechanism has been proposed in a large phase II study [25]. However Sel-P has been shown to be a protein of the negative acute phase response, which makes its induction by Se in septic shock at the early phase debatable [39]. Moreover the first results of the post-peritonitis sheep study did not fit with such an action, without definitive demonstration in the current absence of available Sel-P dosage in sheep [22,23,40]. Many questions on the toxicity limit and mechanism of action, of Na_2SeO_3 are still open [7,16,25–29]. On one-hand doses above the UL and especially in bolus seems more efficient in clinical studies. On the other hand, it is also still commonly thought that, in an oxidative state such as septic shock, Na_2SeO_3 may have toxic side effects at doses above the Upper intake Level (UL) (400 μg), with even more effects above the No Adverse Effect Level (NOAEL) (800 μg) [16,27,28,41–44]. Until now, we had no precise indication about toxicity limits of Na_2SeO_3 in septic condition despite numerous clinical studies performed without preclinical data [7,16,25–29]. These preclinical toxicology data are needed to determine the safety margin of Na_2SeO_3 injection in sepsis patients [7]. This study was conducted to test in a septic shock animal model the double hypothesis that: (i) high dose of pentahydrate Na_2SeO_3 ($5H_2O{\cdot}Na_2SeO_3$) has an increased toxicity in septic condition, and to have indication about safety margins (ii) and Na_2SeO_3 may also have a beneficial pharmacological effect at doses currently considered to be toxic due to its oxidant effect [8,17,18].

Materials and methods

In the present study, we used a common model of acute inflammation related to sepsis by intraperitoneal (IP) injections of lipopolysaccharide (LPS) in rats [45–47]. IP injection of Na_2SeO_3 in rat is also a common model to test its toxicity [9,48,49]. We conducted a three-step study to test the double hypothesis. In the first step, we evaluated the toxic effects of Na_2SeO_3 and of LPS. We determined the 70% lethal dose (LD) for LPS and the Minimum Dose Without Toxic effect for Na_2SeO_3 (MDWT). In the second step, we induced sepsis by injecting the previously determined 70% LD for LPS followed one hour later by the Na_2SeO_3 at the MDWT to determine if Na_2SeO_3 could have therapeutic effects at nearly toxic dose. In a third step we induced similarly sepsis and observed mortality after Na_2SeO_3 IP injection at doses higher than the MDWT.

Animals

Animal housing and experimental procedures were approved by the Ethics Committee on Animal Research of the French Armed Forces Biological Research Institute (IRBA), in accordance with the French ethical guidelines under the number 50/2001. All experiments were conducted on 9-week-old male IOPS Han Ico Wistar rats (Charles River, L'Arbresle, France). Animal weight was between 240 and 280 g at the experimentation time. They were housed in individual confinements in thermoformed polystyrene cages in accordance with standards accredited by the French Ministry of Agriculture and Environment at a temperature of $21 \pm 1\,^{\circ}C$, with 55% relative humidity and a 12 h light–dark cycle. They had free access to food (SAFE, Augy, France) containing 0.190 mg Se/kg of food and to water corresponding to an adequate Se intake [50]. To minimize experimental stressors, rats where acclimatized to the laboratory environment for one week prior to experiments.

Experimental protocol

This study was divided into three distinct steps involving 204 rats. They were conducted to evaluate the effects of an IP injection of $5H_2O{\cdot}Na_2SeO_3$: firstly in healthy rats allowing to determine the MDWT, secondly in rats with an endotoxin shock induced by LPS administrated IP at doses corresponding to the previously determined MDWT doses and thirdly at higher $5H_2O{\cdot}Na_2SeO_3$ doses. In each experiment the rats received two IP injections at 1-h interval (see Fig. 1). When administered, the same LPS dose (26 mg/kg) was used in all experiments. This dose was previously determined in our laboratory as the dose inducing between 60 and 70% mortality (70 LD) during the first 48 h following IP injection of LPS (data not shown) (26 mg/kg of LPS) (*Salmonella typhimurium*, Sigma–Aldrich, France). A 70% mortality rate was chosen in order to observe if sodium selenite might increase the mortality related to sepsis, or decrease it at a dose just below the toxic dose. The selenite used was the pentahydrate sodium selenite ($5H_2O{\cdot}Na_2SeO_3$, Fluka, France) diluted with standard sterilized water, which is the form of selenite used in clinical study by us and others [25,26].

First step of the study: intraperitoneal Na_2SeO_3 toxicity in normal rats

The objective of the first set of experiments was to determine in 74 healthy rats the MDWT of Na_2SeO_3 administrated IP, as well as its minimum LD dose (Fig. 1a).

In the 74 rats, we first injected 2.5 mL saline water through IP. One hour later, the rats were divided into seven groups (Fig. 1a). The control rats received a second saline injection ($n = 12$). The 62 other rats were divided into six groups each receiving different doses of Na_2SeO_3 expressed in Se content from 0.25 to 4.5 mg Se/kg (Fig. 1a). For analytical reasons (number of sampled rats among those who survived 48 h), the six groups of rats were grouped together into three groups (1, 2, 3) according to the dose received (expressed in selenium – Se – content). Group 1 received Na_2SeO_3 dose corresponding to 0.25 or 0.35 mg/kg Se (Na_2SeO_3 dose 1, $n = 20$). Group 2 received Na_2SeO_3 dose corresponding to 1 or 1.75 mg/kg Se (Na_2SeO_3 dose 2, $n = 20$) and Group 3 Na_2SeO_3 dose corresponding to 3 or 4.5 mg/kg Se (Na_2SeO_3 dose 3, $n = 22$) content. Na_2SeO_3 was diluted in saline water (1 mL per 100 g of body weight), and injected

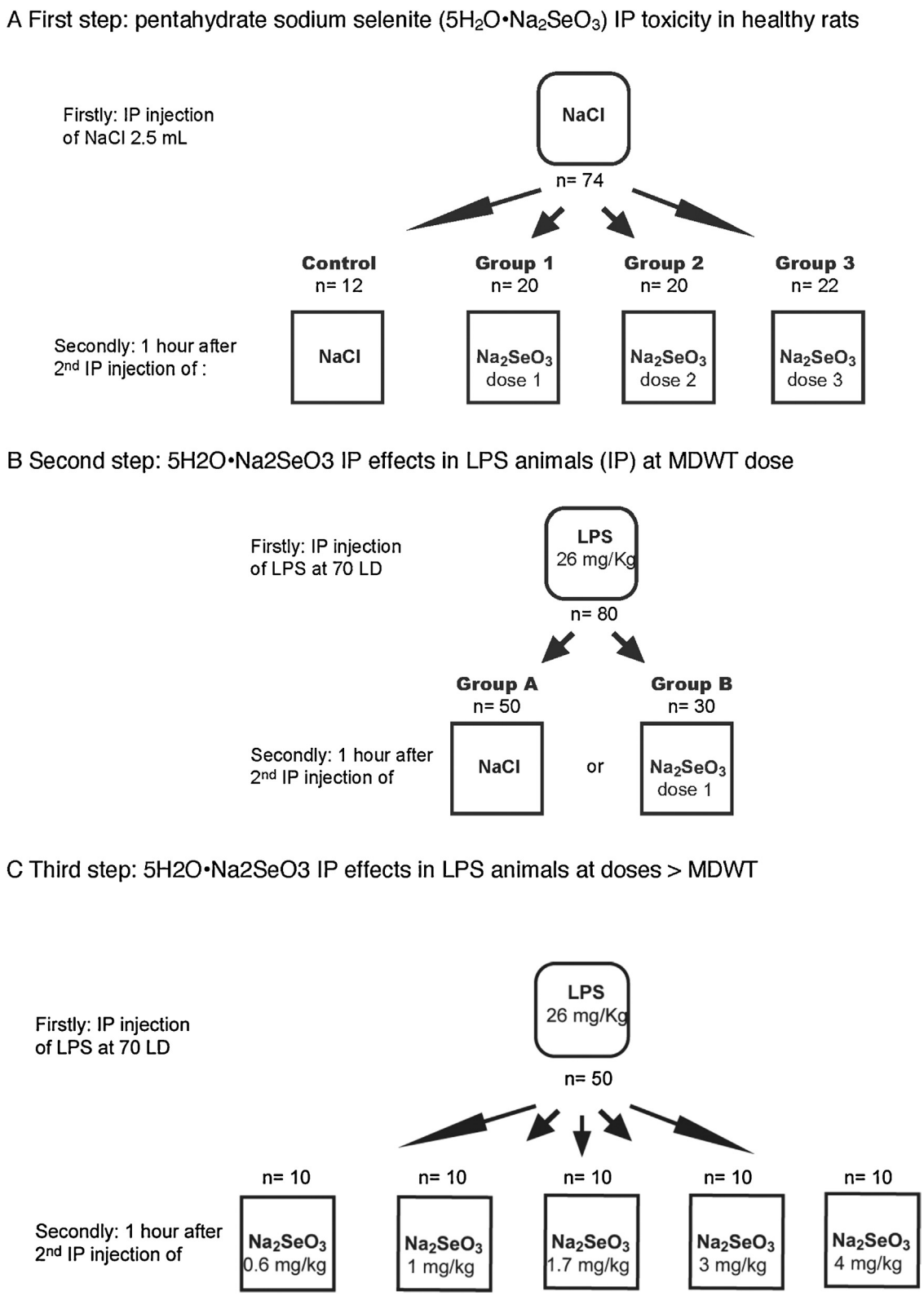

Fig. 1. IP: intraperitoneal, Na_2SeO_3: pentahydrate sodium selenite, LPS: lipopolysaccharide, LD: lethal dose. For both steps, mortality and biological parameters were assessed 48 h after the second injection, MDWT: Minimum Dose Without Toxic effect in healthy animals. Dose 1 corresponded to an administration of pentahydrate sodium selenite ($Na_2SeO_3 \cdot 5H_2O$) expressed in selenium (Se) content of 0.25 or 0.35 mg Se/kg body weight; dose 2 of 1 or 1.75 mg/kg; dose 3 of 3 or 4.5 mg/kg. Dose 1 was determined as the MDWT. Doses in (C) corresponded to an administration of pentahydrate sodium selenite ($Na_2SeO_3 \cdot 5H_2O$) expressed in Se content.

through IP. Clinical and biological evaluations and mortality were assessed 48 h after the second IP injections.

Second step of the study: intraperitoneal Na_2SeO_3 effects in LPS treated rats

Using the results of the first experiment, in the second one we induced septic shock by injection of 70% LD dose of LPS and tested whether the MDWT of Na_2SeO_3 could have a therapeutic effect (Fig. 1b).

In this step 80 rats were tested. They all received IP LPS injection. One hour later, Group A ($n = 50$) received IP saline vehicle (1 mL per 100 g of body weight) (LPS control group). Group B ($n = 30$) received IP Na_2SeO_3 injection (0.25–0.35 mg/kg). As in the first experiment, clinical and biological evaluations and mortality were assessed 48 h after the second IP injections.

Third step of the study: intraperitoneal Na_2SeO_3 effects in LPS treated rats at dose higher than the MDWT

Similarly to the second step, septic shock was induced in rats. We tested whether doses higher than the MDWT had toxic effects.

In this step 50 rats were tested. They all received LPS injection. One hour later, rats received IP Na_2SeO_3 injection at doses 0.6, 1, 1.75, 3 and 4 mg/kg ($n = 10$ each dose) (Fig. 1c).

Biological endpoints

At the end of each 48 h follow-up period, blood samples were drawn in part of the surviving rats of each group, in order to allow biochemical assays. To allow trace element analysis, oligo-free needles, syringes and tubes were used. Blood was collected under halothane anesthesia by vena cava puncture with EDTA and Heparin-Lithium (HL) as anticoagulant and then the rats were killed by sectioning heart vessels. Especially in hypovolemic rats both EDTA and HL, blood sampling could not be always done. After centrifugation at 2000 g for 15 min., plasma samples were stored at −80 °C until analysis. HL plasma levels of creatinine, lactic acid, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured with an automatic analytical instrument (Hitachi 912, Roche Diagnostics, Meylan, France). The values for each sample were evaluated using normal and pathological standards, Précinorm® U and Précipath® U (Roche Diagnostics, Meylan, France). Nitric oxide (NO) plasma concentration was measured in EDTA plasma samples using a specific enzyme-linked immunosorbent assay (ELISA) technique according to the manufacturer's instructions (R&D Systems Europe, Lille, France). The minimum detectable concentration for NO was less than 3.12 μmol/L. Se concentration was measured in HL plasma using capillary gas chromatography–mass spectrometry (TRIO 1000, ThermoQuest, Les Ulis, France) in single ion recording according to Ducros and Favier methodology [51]. As this study was focused on mortality and toxicity no measurement of plasma glutathione peroxidase and selenoprotein-P was performed.

Statistical analysis

Reported values are expressed as means ± standard deviation for quantitative variables or numbers and percentages for qualitative variables. Statistical analysis was performed with SAS statistical software V9.1 (SAS Institute, Cary, NC). Our statistical plan included two distinct analyses. In the first step of the study, biological parameters were compared between the four groups using a one-way analysis of variance or the Kruskal–Wallis Test, when needed. In case of a statistically significant difference, pairwise comparisons were performed using the Student's t test with Bonferroni correction. In the second step of the study, biological parameters were compared between the two LPS groups – with and without selenite administration – using the Student's t test or the Wilcoxon rank sum test when needed. Survival rates at 48 h were compared using the chi-square test. In each case, a p value <0.05 was considered statistically significant.

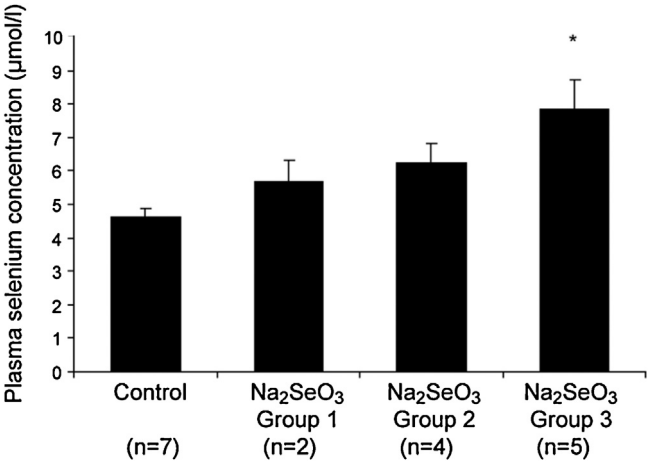

Fig. 2. Data are means ± standard deviations. In each group, the number of sampled surviving rats is indicated in bracket. Na_2SeO_3 (sodium selenite) injected IP in Groups 1, 2 and 3 correspond to 0.25 or 0.35 mg/kg, 1 or 1.75 mg/kg, and 3 or 4.5 mg/kg Se content respectively; $p < 0.001$ (ANOVA) between the 4 groups. *$p < 0.05$ vs Control (Bonferroni correction).

Results

First step of the study

For doses of Se greater than 1 mg/kg, rats had typical neurological acute toxicity symptoms such as transient wandering difficulties, especially with hind legs. Some of them presented also a clinical aspect of encephalopathy, lying on their backs and even eating on their backs.

Biological variables measured in the first experiment are shown in Table 1 and Fig. 2. Mean plasma creatinine concentrations increased significantly in animals receiving Na_2SeO_3 doses 2 and 3 as compared to the control group (+104% with Na_2SeO_3 dose 2 and +126% with Na_2SeO_3 dose 3, $p < 0.05$) and also as compared to the Na_2SeO_3 dose 1 group (+96% with Na_2SeO_3 dose 2 and +118% with Na_2SeO_3 dose 3, $p < 0.05$). Mean plasma AST and ALT concentrations were significantly higher in animals receiving Na_2SeO_3 dose 3 as compared to all other groups. No significant differences between groups were observed for lactic acid and NO plasma concentrations. Compared to the control group, plasma Se concentration was significantly increased only with Na_2SeO_3 dose 3 (Fig. 2).

No rats died within 48 h neither in the control group nor in the two groups of animals receiving the four smallest Se doses (0.25, 0.35, 1 and 1.75 mg/kg). In the Na_2SeO_3 dose 3 group, 4/22 animals died within 48 h (18% mortality, [95% confidence interval: 2–34%]).

Second step of the study

Rats receiving IP 26 mg/kg dose LPS alone in 3 mL water (Fig. 1B) rapidly showed signs of severe sepsis in a few hours. They rolled up into a ball. Their fur was dull and stood on end. They were prostrated, asthenic and had diarrhea. LPS non-surviving rats died mostly in less than 24 h in an asthenic syndrome and LPS alone surviving rats remained very asthenic at 48 h. Although not quantified and a subjective observation, Na_2SeO_3 treated rats seemed to be healthier showing less prostration and asthenia.

Plasma Se concentrations increased at 48 h in animals receiving Na_2SeO_3 dose 1 (Fig. 3) compared to those receiving LPS alone.

Table 1
Organ dysfunction biological variables in the control and Na_2SeO_3 treated rats.

Variable	Control ($n=12$)	Na_2SeO_3 Group 1 ($n=14$)	Na_2SeO_3 Group 2 ($n=4$)	Na_2SeO_3 Group 3 ($n=5$)	p
Creatinine (μmol/L)	27 ± 12[d]	28 ± 13[d]	55 ± 6	61 ± 22	0.001
AST (UI/L)	60 ± 7	88 ± 27	89 ± 18	531 ± 321[e]	<0.001
ALT (UI/L)	35 ± 7	41 ± 9	30 ± 11	80 ± 39[e]	0.03
Lactic acid (mmol/L)	3.39 ± 0.18[a]	4.45 ± 1.91[a]	3.05 ± 0.30	4.28 ± 1.91[b]	NS
NO (μmol/L)	21 ± 7[c]	27 ± 10[a]	29 ± 12	24 ± 10[b]	NS

Data are means ± standard deviations.
Na_2SeO_3 (sodium selenite); doses 1, 2 and 3 correspond to 0.25–0.35 mg/kg, 1–1.75 mg/kg, and 3–4.5 mg/kg selenium content; AST, aspartate aminotransferase; ALT, alanine aminotransferase ALT; NO, nitric oxide.
[a] $n=2$.
[b] $n=4$.
[c] $n=6$.
[d] vs Na_2SeO_3 doses 2 and 3 (Bonferroni correction).
[e] vs Control and Na_2SeO_3 doses 1 and 2 (Bonferroni correction).

Plasma Se concentration decreased in LPS treated animals as compared to the control animals of the first step (4.62 ± 0.24 μmol/L in controls and 3.50 ± 0.50 μmol/L with LPS, $p=0.001$). Na_2SeO_3 dose 1 supplementation to LPS treated animals restored plasma Se concentrations to normal values (4.27 ± 0.65 μmol/L, $p=0.157$ vs. controls of the first step).

Plasma NO concentration significantly decreased in animals receiving Na_2SeO_3 (Fig. 4A, −31%, $p=0.038$) compared to those receiving LPS alone. Although non significant, plasma lactic acid concentration also tended to decrease (Fig. 4B, −19%, $p=0.068$). Plasma lactic acid concentrations increased in LPS treated animals compared to control animals of the first step (3.39 ± 0.18 in controls and 4.81 ± 0.80 with LPS, $p=0.045$). Na_2SeO_3 dose 1 supplementation in LPS treated animals restored values similar to normal values (3.88 ± 0.72, $p=0.314$ vs controls of the first step). No significant differences between groups were observed for creatinine, AST and ALT plasma concentrations (data not shown).

Mortality at 48 h was 37/50 (74%, [95% confidence interval: 62–86%]) with LPS alone and 20/30 (67%, [95% confidence interval: 50–84%]) in the LPS animals receiving Na_2SeO_3 ($p=0.483$).

Third step of the study

All animals (50/50) receiving doses equal or higher than 0.6 mg/kg $5H_2O{\cdot}Na_2SeO_3$ died before 48 h follow-up period, mainly after a 5 h clinically free period interval from acute respiratory distress associated to tinged with blood sputum, suggesting an acute pulmonary edema. This mortality rate is significantly increased compared to those of rats receiving LPS only (37/50), or LPS followed by $5H_2O{\cdot}Na_2SeO_3$ at MDWT (20/30), $p<0.001$. Samples could be obtained in one animal close to its death in the 0.6 mg/kg group. Low blood volume could be obtained. Therefore, only the following

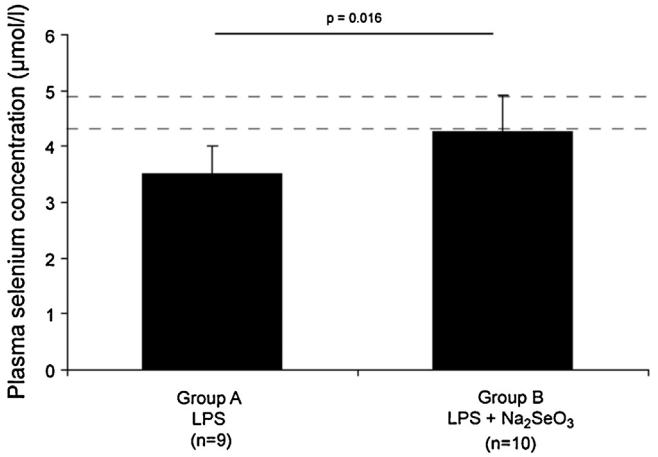

Fig. 3. Data are means ± standard deviations (SD). In bracket are indicated the number of sampled rats among the surviving rats at 48 h. Mean ± SD values in control rats are indicated as doted lower and upper lines ($n=7$). LPS (lipopolysaccharide); Na_2SeO_3 (sodium selenite); Na_2SeO_3 administrated doses in Group B correspond to 0.25–0.35 mg Se/kg (dose 1).

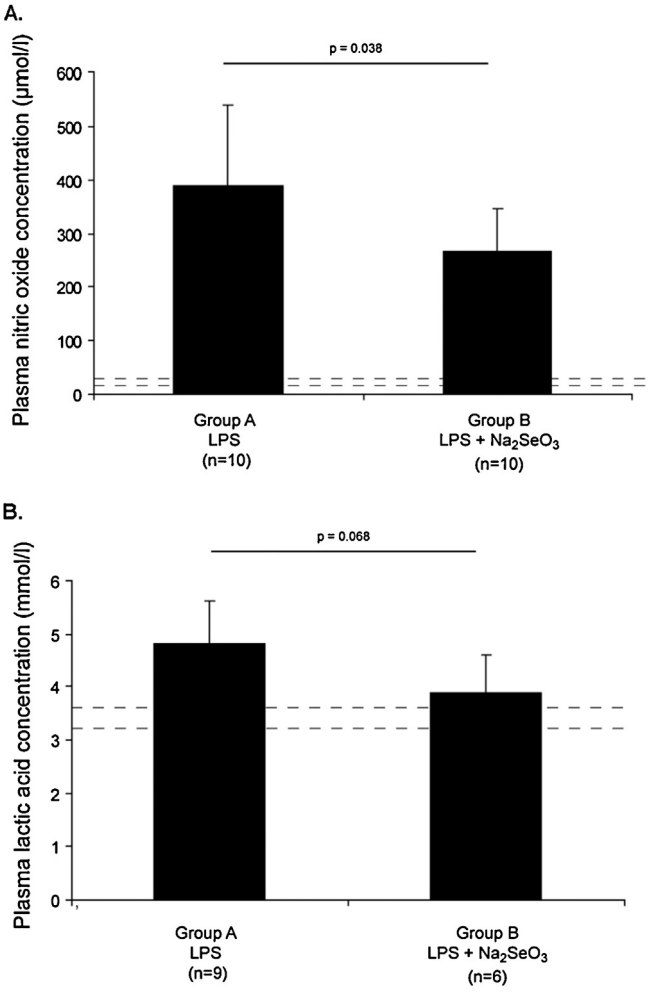

Fig. 4. Data are means ± standard deviations (SD). In bracket are indicated the number of sampled rats among the surviving rats at 48 h. LPS (lipopolysaccharide); Na_2SeO_3 (sodium selenite); LPS (lipopolysaccharide); Na_2SeO_3 (sodium selenite); Na_2SeO_3 administrated doses in Group B correspond to 0.25–0.35 mg Se/kg (dose 1). Values for nitric oxide (NO) concentration were 283 μmol/L for Na_2SeO_3 dose corresponding to 0.25 mg Se/kg and 256 μmol/L for 0.35 mg Se/kg respectively. Mean ± SD values for (NO) ($n=6$) and for lactate acid ($n=2$) in control rats are indicated as lower and upper doted lines.

parameters were obtained: creatinine 77 μmol/L, AST 131 UI/L, ALAT 51 UI/L, plasma Se concentration 13.9 μmol/L.

Discussion

This is the first study that evaluates in the same animal model the IP toxicity of pentahydrate sodium selenite ($5H_2O{\cdot}Na_2SeO_3$), a small Se-containing compound, and the IP toxicity of lipopolysaccharide (LPS) at 70% LD and to determine IP $5H_2O{\cdot}Na_2SeO_3$ toxicity in septic shock animals in a post-LPS model. Even though high doses of Na_2SeO_3 and LPS are both known to lead to multiple organ failure and death caused by pathophysiological mechanisms involving hyperoxidation, their mechanisms differ significantly. This is shown by the absence of significant increase of plasma NO and lactate concentration in selenite acute intoxication contrary to their marked increase in LPS toxicity.

The minimum LD dose for Na_2SeO_3 was lower in septic compared to healthy rats (lower than 0.6 mg/kg vs 3 mg/kg Se as $5H_2O{\cdot}Na_2SeO_3$). This is in favor of an increased toxicity of $5H_2O{\cdot}Na_2SeO_3$ in septic condition. However, IP administration of Na_2SeO_3 at Minimum Dose Without Toxic effect (MDWT) dose (0.25–0.35 mg/kg Se) one hour after administration of 70% LD of LPS may be beneficial as shown by the decrease of NO concentration compared to animal receiving LPS only.

Rats: a resistant to oxidation model, possible link with plasma selenoproteins

In accordance with previous finding, plasma Se concentration in healthy rats was five times higher than in man [52,53]. This may allow rats to support a higher hyperoxidation than man and may partially explain rats' higher resistance to infection compared to men [54,55]. Similar to the decrease in plasma Se observed in ICU patients, we observed a decrease in plasma Se concentration in surviving LPS rats [22,50,52,56,57].

Margin of safety of Na_2SeO_3

Among seleno-compounds, Na_2SeO_3 is a relatively high oxidant selenocompound [10,17] and therefore listed as highly toxic [11]. One can notice that Na_2SeO_3 is as toxic as arsenic salt in rat in IP injection [48]. In rat, the 50% LD of Se, which is physico-chemically close to oxygen [10], varied: from 2.5 mg Se/kg if administrated as Na_2SeO_3 to 6.7 g Se/kg if administrated as elemental Se [43,58]. Our results for a minimum LD of 3 mg Se/kg for Na_2SeO_3 in healthy rat are in accordance with previous reports on mortality, as are our results on renal and hepatic toxicity already described in healthy mammals including men in acute intoxication or poisoning [9,48,49,59,60]. In our study, the kinetic of plasma Na_2O_3Se after IP injection following IP LPS administration may be more likely similar to a continuous administration resulting in a plateau than an intravenous (IV) bolus injection resulting in a peak of concentration [22]. IP is therefore likely to be less toxic than IV bolus administration [9,10,49,59]. We nevertheless observed a marked increased toxicity of Na_2SeO_3 in LPS compared to healthy rat, which is in accordance with the increased cytotoxicity of Na_2SeO_3 in activated cells compared to non-activated cells [13,21].

In humans, many authors believe that in such an oxidative state as septic shock, Na_2SeO_3 has toxic side effects at doses above the nutritional limits UL (400 μg/day) or the NOAEL (800 μg) [16,27,28,41–44]. On the contrary, other authors believe that higher doses than NOAEL are required for Se supplementation [6,25,30,61]. Very few support Na_2SeO_3 acting as cytotoxic drug, especially when administrated in bolus, such as has been proposed in cancer [7,10,19,21,61,62]. In the last two cases, with Na_2SeO_3 administration at high doses, determination of the security margin is required. In septic shock ICU patients mortality tends to decrease in clinical studies using Na_2SeO_3 in bolus at doses largely above UL [6,16,25], but not in those were it is administrated continuously [16,25,26,63]. In a post-peritonitis sheep study, bolus was needed for efficiency, supporting an oxidant drug action [22,23,40]. For the use of Na_2SeO_3 as a chemotherapeutic agent, a phase I study has been conducted in stabilized drug resistant cancer patients using bolus administration of Na_2SeO_3. This study was presented recently in the 10th International Symposium on Selenium [64]. According to the authors the toxicity limit of IV bolus injection in stable patients was 10.2 mg Se/m^2 (about 0.25 mg/kg) [64] which is less than the one estimated in ingestion (about 3 mg/kg) [9]. Our study supports that the margin of safety of Na_2SeO_3 be also further restricted in septic shock compared to stable condition.

Oxidant beneficial effect of Na_2SeO_3 acting as drug in sepsis?

As an illustration of the close link between a drug and a poison, and the need to determine the safety margin, the IP Na_2SeO_3 injection at 0.25–0.35 mg Se/kg seemed beneficial, when its administration at 0.6 mg Se/kg was lethal.

In accordance with our hypothesis, we observed, a NO reduction at this IP dose of Na_2SeO_3 administrated after the LPS injection compared to LPS rat. This is in accordance with in vitro studies where large concentrations of Na_2SeO_3 result in reversible inhibition of NO synthesis [20,65,66], or a cytotoxic effect observed in activated cells such as cancer cells [67,68]. This cytotoxic action will require to reach a blood concentration known to be toxic for cells in vitro, and especially on non-adherent cells (>2–5 μmol Se/L as Na_2SeO_3) [8,10,17,18,21]. It may be beneficial in sepsis by reducing the activity of over-activated phagocytic cells [7,8,17,18] and may explain the results obtained in clinical studies when Na_2SeO_3 is injected in bolus [7]. The trend of reduced lactate concentrations is also in accordance with results of the post-peritonitis sheep study, which support an oxidant cytotoxic drug action of Na_2SeO_3, that requires to reach a high level of plasma Na_2SeO_3 concentration (>2–5 μmol Se/L). This concentration was only obtained in bolus in this study where Na_2SeO_3 was administrated after the onset of peritonitis [22]. Contrary to stable condition the cells and especially the circulating phagocytic cells are over-activated in septic shock. In this condition, the administration of Na_2SeO_3 is more likely to act through its cytotoxicity [10,13,15,21]. An induction of selenoprotein synthesis by Na_2SeO_3 is unlikely in over-activated condition contrary to non-activated one [21] and has never been studied at the early hours after injection in a post-sepsis model. In addition, the main plasma selenoprotein (selenoprotein-P) belongs to the negative acute phase response of septic shock [39].

Limitations

This rat study did not allow to conclude if the observed reduction of NO is related to an oxidative effect of high dose Na_2SeO_3 or to an anti-oxidant indirect effect through Se inducing selenoenzymes, such as GPx or selenoprotein-P, synthesis [25,40,66,69]. Further studies are required to determine this, with selenoprotein-P and plasma GPx dosages during the first hours following Na_2SeO_3 injection [23,40]. This study has several additional limitations for transposition to ICU patients. Firstly, regarding the animal model, as in all studies with rats, we recruited young, healthy rats weighting 300 g, for an average weight of 800 g for an adult. In addition, only male rats were selected. Secondly, concerning the supportive therapies, the animals did not receive sedation, mechanical ventilation, vasopressor therapy, active fluid resuscitation or antibiotics that may be required in cases of bacterial translocation. Thirdly there was no follow-up of hemodynamic nor pulmonary function. Fourthly, since the reference range for plasma Se is 5 times higher

than in man, and if selenoprotein-P has a major antioxidant function, rats may be more tolerant to very high intakes of oxidant doses of Na_2SeO_3 than humans [54,55,70]. Fourthly, sampling was limited to part of the surviving animals limiting the power of statistical analysis especially for lactate concentration. Fifthly, this model was essentially performed to evaluate the security margin of continuous administration, but not for the more dangerous intravenous bolus administration allowing higher selenite blood concentration than IP administration. On the other hand, regarding the beneficial effect of oxidant Na_2SeO_3 dose, which was not the main objective of this study, other parameters than plasma Se, lactate and NO concentration should be studied further to precisely understand the mechanism of action.

Conclusion

Even though high doses of sodium selenite (Na_2SeO_3) and LPS are both known to lead to multiple organ failure and death caused by pathophysiological mechanisms involving hyperoxidation, their mechanisms seem to differ significantly as shown by their markedly different effects on NO and lactate concentrations observed in this study. In our study the toxicity of IP administration of sodium selenite increased in septic shock rats compared to healthy rats, which are especially resistant animals to aggression. Toxicity of sodium selenite may be higher in septic shock ICU patients than in rats. Moreover, our model is more similar to a continuous administration than to an intravenous bolus administration, which is believed to cause higher toxicity. In addition, this study did not explore long-term toxicity.

Further studies are required to be more precise about (i) the mechanism of action of Na_2SeO_3, especially just after a bolus injection. Na_2SeO_3 could act as an oxidant cytotoxic drug (especially on hyper-activated circulating phagocytic cells) or as Se supplier for antioxidant selenoenzymes such as GPx or selenoprotein-P synthesis; (ii) rodent and non-rodent septic shock animal studies are also required, to determine more precisely Na_2SeO_3 toxicity limit in sepsis. The publication of the results of the previously cited phase I study on Na_2SeO_3 bolus administration as second line chemotherapy in resistant cancer may be of great interest for safety margin determination, taking into account the increased toxicity of Na_2SeO_3 in sepsis shown in our study.

Conflict of interest

The corresponding author to be co-inventor of patent FR 98 10889, PCT N°FR 99/02.66 (delivered: US 6,844,012 B1, Au 760 534; EP1107767) on the "use of Selenium for Treating Systemic Inflammatory Response Syndrome (SIRS), and Composition for implementing said Treatment", and also main shareholder of an early stage start-up named SÉRÉNITE-Forceville committed to early diagnostic and treatment of septic shock. This patent pertains to, without being limited to, the use of at least one molecule comprising selenium, especially selenite, in an amount corresponding to a daily dose of about 2–80 mg of atomic selenium equivalent, for the production of a drug intended for the treatment of severe systemic inflammatory syndrome especially in the case of severe acute infectious state. However, neither did SÉRÉNITÉ-Forceville nor did the author receive any payment for this patent from any company.

All the other authors declare to have no conflict of interest.

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